Reverse smoking is a common practice in some parts of India, whereby the lighted end of a homemade cigar is held inside the mouth. Pindborg, et al. (*64*) conducted an epidemiological survey of 10,169 villagers in the Srikakulam district of south India and found that 43.8 percent of those interviewed practiced reverse smoking. Leukoplakia was found in 8.8 percent of reverse smokers compared to 0.1 percent in nonsmokers. The 10 patients found to have oral cancer were all reverse smokers. Reddy, et al. (*68*) found that reverse smoking was practiced by 73 of 100 patients with oral cancer. Reddy, et al. (*66*, *67*) reported characteristic histologic findings of the oral cavity in biopsies obtained from reverse smokers. In two other studies from India, changes in the ultrastructure of the oral mucosa of chewers (*54*) and smokers (*65*) are described.

## Cancer of the Esophagus

In the Japanese prospective study, Hirayama (*37*) reported that male smokers had a mortality ratio for cancer of the esophagus of 2.24 compared to nonsmokers ($P<0.001$). Martinez (*57*) studied the relationship between smoking in various forms and the development of cancer of the esophagus in a retrospective study of 179 patients. Dose-response relationships were demonstrated for the amount smoked and alcohol consumption and the development of cancer of the esophagus.

## Cancer of the Larynx

The mortality ratios for cancer of the larynx in the large Japanese prospective study were reported by Hirayama (*37*) to be 11.0 for male cigarette smokers and 9.0 for female cigarette smokers compared to nonsmokers ($P<0.001$).

Stell (*85*) conducted a retrospective study of 190 patients with carcinoma of the larynx. Only 13 percent of the patients were nonsmokers or ex-smokers compared with 41 percent of the controls. The relative risk ratio for heavy cigarette smokers was 3.48 compared to nonsmokers. The relative risk was 1.34 for smokers of pipes and cigars.

Moore (*59*) reported the occurrence of second primary cancers in 203 smokers who had been surgically treated for cancer of the oral cavity, pharynx, or larynx, without recurrence for a period of 3 years.

Within an average followup period of 7 years, 40 percent of the 122 patients who continued to smoke developed second primary cancers of the upper respiratory or digestive tract, but only 6 percent of the patients who stopped smoking developed second cancers. A total of 50 patients with cancer of the larynx underwent laryngectomy. Of the 16 who continued to smoke, three developed a second cancer, whereas none of the 34 ex-smokers without a larynx developed a second primary malignancy.

## Cancer of the Pancreas

Hirayama (*37*) reported a significant association between cigarette smoking and the development of cancer of the pancreas. The mortality ratios were 2.05 ($P<0.001$) for men and 1.9 ($P<0.05$) for women.

Krain (*47*) reviewed a number of environmental factors that may be associated with the 15 percent annual increase in the death rate from cancer of the pancreas found in the United States. The strongest associations appeared to be with cigarette smoking and certain occupational exposures.

## Cancer of the Kidney and Urinary Bladder

Hirayama (*37*) reported a mortality ratio of 2.71 for cancer of the kidney and bladder in women who smoke cigarettes ($P<0.001$). The mortality ratio of 1.07 for men who smoked compared to nonsmokers was not significant; however, the few deaths from this cancer among men in the Japanese study did not allow conclusions to be drawn.

Hoover and Cole (*39*) examined the strength of the association between cigarette smoking and the development of bladder cancer in successive birth cohorts of men and women in the United States, Denmark, England, and Wales. Increasing rates of bladder cancer were observed in populations characterized by an increase in cigarette smoking among successive birth cohorts. The association was consistent in both men and women, and was also consistent for different nationalities and urban and rural groups. These findings suggest a causal role for cigarette smoking in the development of bladder cancer.

In a retrospective study from Germany, Fischer (*27*) examined the smoking habits of 162 men with bladder cancer and a control group of 198 men who had benign prostatic hypertrophy. The relative risk

ratio was 6.4 for smokers of fewer than 15 cigarettes a day, and 27.5 for smokers using more than this amount. Only 3 percent of the men with bladder cancer were nonsmokers.

Xipell (*103*) studied renal nodules in 250 patients in Australia who came to autopsy. Benign adenomas were the most common lesions and were found in 22 percent of the patients. The remaining nodules were cysts, thrombosed veins, abscesses, granulomas, and metastatic lesions. A statistically significant difference between the smoking habits of those with adenomas and those with the miscellaneous lesions was reported ($P<0.012$). All the adenomas were found in smokers.

Cole, et al. (*14*) conducted a retrospective study of 461 persons with transitional or squamous cell carcinoma of the lower urinary tract. After the data were controlled for cigarette smoking, occupational exposure appeared to contribute to 18 percent of the lower urinary tract cancer among men aged 20 to 89 compared to the 39 percent attributed to cigarette smoking in men in a previous report (*15*).

Werf-Messing and Kaa'en (*100*) examined the association of occupational exposure and smoking in the development of bladder cancer in 346 males in the Netherlands who had this disease. The smoking habits of cancer and control patients in each group were nearly identical; however, patients with bladder cancer had a longer exposure to hazardous working conditions than did controls.

## Experimental Carcinogenesis

Experimental studies, mainly in animals, have added to an understanding of many of the processes involved in tobacco carcinogenesis. Possible mechanisms of chemical carcinogenesis were reviewed by Miller and Miller (*58*), Ryser (*76*), and Leone (*51*). Electron spin resonance studies of carcinogenesis were reviewed by Swartz (*87*). Franke (*28*) discussed the posible role of hydrophobic interactions of polycyclic aromatic hydrocarbons with protein in chemical carcinogenesis. Chemical carcinogenesis in Syrian hamsters was reviewed by Shubik (*82*) and Homburger (*38*).

### *Respiratory Tract Carcinogenesis*

Epidemiological, clinical, and autopsy data from studies of humans have established cigarette smoking as the major cause of lung cancer in the United States. One of the reasons it has not been possible to

characterize fully the mechanisms responsible for this causal relationship is the lack of an ideal animal model in which to study respiratory tract carcinogenesis in the laboratory. Exposing animals to cigarette smoke in a closed chamber does not replicate the kinds of exposure smoking humans receive, although some recently developed smoking chambers provide conditions similar to the exposure experienced by human smokers. Many animals are obligatory nose breathers and, in them, a large portion of the particulate phase of cigarette smoke may be removed by turbulent precipitation in the nasal passages or larynx before reaching the sites in the lung most commonly exposed in humans. Auerbach, et al. (*3*) first demonstrated that malignant lung tumors could be produced in smoking dogs who were taught to smoke through a tracheostoma. Several investigators have recently examined respiratory tract carcinogenesis in animals using intratracheal instillations of chemical carcinogens found in cigarette smoke, including benzo(a)pyrene and 7H-dibenz(d,g)carbazole. Tumors resulting from this type of treatment are frequently similar to lung tumors found in humans (*24, 32, 33, 36, 77, 80*).

Harris, et al. (*33*) examined the acute ultrastructural effects of benzo(a)pyrene carried on ferric oxide particles on the tracheobronchial epithelium of the Syrian Golden hamster. Test substances were administered by intratracheal instillation. Ferric oxide alone resulted in some focal replacement of columnar epithelium with polygonal basal cells. This effect was reversed by termination of the treatment. After treatment with benzo(a)pyrene and ferric oxide, focal replacement of the columnar cells with pleomorphic cells occurred. These pleomorphic cells had the ultrastructural features of atypical squamous cells and were similar to the hyperplastic epithelial cells described in the bronchi of smoking dogs and the neoplastic squamous cells found in human bronchogenic carcinoma.

In an extension of this study, Harris, et al. (*32*) reported that vitamin A deficiency or the application of benzo(a)pyrene-ferric oxide through intratracheal instillation resulted in squamous metaplasia of the trachea. Both lesions appeared to be morphologically similar by light microscopy, but at the ultrastructural level significant differences were observed. Squamous metaplasia induced by benzo(a)pyrene-ferric oxide was characterized by defects in the basement membrane, enlarged nuclei with cytoplasmic invaginations, and pleomorphic nucleoli not seen following vitamin A deficiency.

Sellakumar and Shubik (*80*) treated Golden Syrian hamsters with weekly intratracheal instillations of 7H-dibenz(c,g)carbazole (7H-DBC) suspended with equal amounts of ferric oxide in a saline solution. One group of 35 hamsters was treated with 45 mg. of the carcinogen and a second group was treated with 15 mg. More than 85 percent of the animals in each group developed respiratory tract

tumors. Most of the tumors occurred in the major airways and were squamous cell carcinomas. Adenocarcinomas and anaplastic carcinomas were found less frequently.

Saffiotti, et al. (*77*) examined the carcinogenic effects of benzo(a) pyrene prepared as a suspension of fine crystalline particles attached to ferric oxide in a physiologic saline solution and administered by intratracheal applications to Syrian Golden hamsters. Various concentrations of benzo(a)pyrene and ferric oxide were used in single and multiple applications. A single administration of 37.5 mg. of benzo(a)pyrene with 12.5 mg. of ferric oxide resulted in five bronchogenic carcinomas and five histologically benign respiratory tumors in a total of 61 hamsters. Following multiple administrations, bronchogenic carcinomas including anaplastic and squamous cell types were induced in all dosage groups and a positive dose-response relationship was demonstrated.

Feron (*24*) studied respiratory tract tumors in Syrian Golden hamsters following tracheal instillations of furfural and/or benzo(a) pyrene. Of the 62 hamsters, 41 developed respiratory tract tumors of which squamous cell carcinoma of the trachea was the most frequent type observed. Furfural in combination with benzo(a)pyrene resulted in a higher yield of tumors than was seen with benzo(a)pyrene alone. Furfural alone possessed no carcinogenic activity.

Shabad (*81*) and one of his collaborators, Yanysheva, produced benign and malignant epidermoid lung tumors in rats following single and multiple administrations of benzo(a)pyrene by intratracheal instillation. Dose-response relationships were demonstrated.

### *Experiments in Mice*

Cigarette smoke condensate (CSC), various fractions of CSC, and many chemical compounds identified in CSC have been tested for tumorigenic activity in mice by a variety of methods, including skin painting and subcutaneous injections. Complete carcinogens and incomplete carcinogens, which include tumor initiators, tumor promoters, and tumor accelerators have been described. Several recent studies have been conducted using mice as the experimental animal which examine further the mechanisms involved in tobacco carcinogenesis.

Lee and O'Neill (*50*) measured the effect of duration and dosage of benzo(a)pyrene applications on the rate of development of benign and malignant skin tumors in mice. The incidence rate for tumor formation was directly proportional to both time and dose. These data conformed quite closely to postulated mathematical models of the rate of tumor development.

Davies and Whitehead (*17*) studied the effect of altering the "tar" and nicotine ratio of cigarettes on experimental carcinogenesis. No significant difference in tumor yield was found between condensates obtained from the smoke of cigarettes containing 16.6 mg. "tar" and 1.79 mg. nicotine and other cigarettes containing 10.0 mg. "tar" and 1.94 mg. nicotine.

Several studies by Bock, et al. (*7*, *8*, *9*) have examined the tumor promoting activity of a number of fractions of cigarette smoke condensate (CSC). A number of subfractions of the neutral fraction of CSC were tested for tumor promoting activity in mice pretreated with 7,12-dimethylbenz(a)anthracene as a tumor initiator (*8*). The most polar subfractions and the fraction containing benzo(a)pyrene were the most active tumor promoting fractions. In another study (*9*), the weak acid fraction of CSC was found to be a very weak complete carcinogen which probably acts primarily as a tumor promoting agent. The promoting activity depended primarily on the nonvolatile constituents of this fraction. More recently, Bock, et al. (*7*) reviewed the tumor promoting effects of CSC and extracts of tobacco leaves. A combination of two subfractions of the tobacco extracts, as well as five major fractions of CSC, were found to have tumor promoting activity. The fraction containing the polynuclear aromatic hydrocarbons was found to be a complete carcinogen. Two subfractions were found to be strongly synergistic in their tumor promoting activity when applied simultaneously to mouse skin.

Lazar, et al. (*49*) found that hydroquinone applied to mouse skin in conjunction with the active fractions of CSC accelerated the early histologic changes that result from the application of "tar" or its fractions.

Van Duuren, et al. (*97*) have suggested that "cocarcinogenesis" be differentiated from "tumor promotion" defining "cocarcinogenesis" as the production of malignant tumors by two or more agents applied simultaneously or alternately in single or repeated doses to mouse skin and "tumor promotion" as a single treatment with one agent followed by single or repeated treatment with a second agent. Using these definitions, the authors found several tumor promoting agents to possess cocarcinogenic activity.

Roe, et al. (*74*) studied mechanisms of mouse skin carcinogenesis using benzo(a)pyrene and a neutral fraction of CSC applied singly or in various combinations with each other. Skin tumor incidence rates increased with the dose of applied material for both the neutral fraction and benzo(a)pyrene. Mixtures of the neutral fraction with benzo(a)pyrene did not act independently in the production of malignant skin tumors but synergistically, suggesting that some of the components of the neutral fraction act as cocarcinogens rather than as complete carcinogens.

Schmähl (*78*) found a direct relationship between the dosage and duration of subcutaneous injections of tobacco smoke condensates and the development of sarcomas in rats.

Maenza, et al. (*56*) studied the effects of a combination of nickel subsulfide ($Ni_3S_2$) and benzo(a)pyrene on sarcoma induction in rats. The interval between administration of the carcinogen and the development of sarcomas was significantly shorter ($P<0.001$) in male Fischer rats given injections of a combination of 10 mg. of $Ni_3S_2$ and 5 mg. of benzo(a)pyrene than in rats given either ingredient alone. There appeared to be a synergistic interaction between nickel compounds and the polycyclic aromatic hydrocarbons.

Healey, et al. (*34*) added further refinements to a technique for measuring the nonspecific esterase activity of mouse skin following applications of various chemical compounds. With few exceptions, changes in esterase activity reflected the known tumor producing activity of a number of polycyclic hydrocarbons and tobacco condensates.

Sydnor, et al. (*89*) examined the effect of an aqueous extract of cigarette smoke condensate on benzo(a)pyrene-induced sarcoma in female Sprague-Dawley rats. Benzo(a)pyrene was injected subcutaneously in various concentrations of 12.5 μg. to 400 μg. per dose dissolved in sesame oil. Injections were given on alternate days for 30 doses. The mean tumor induction time was accelerated in five of seven groups given the aqueous extract of CSC in their drinking water. Animals given any benzo(a)pyrene eventually developed sarcomas at the site of injection. Dose-response relationships were demonstrated for the concentration of benzo(a)pyrene administered. It appeared that aqueous extracts of CSC contained one or more components which functioned as cocarcinogens.

### *Aryl Hydrocarbon Hydroxylase (AHH)*

Certain of the chemical compounds found in the gas and particulate phase of cigarette smoke are absorbed through the lung or oral cavity into the general circulation. Possibly through such absorption some chemical carcinogens are carried to target organs not directly exposed to cigarette smoke. Some of these chemical compounds are probably excreted unchanged while others are metabolized to various degrees by enzyme systems present in the liver and many other tissues. The microsomal mixed-function oxidases are key enzyme systems for the metabolism of a wide variety of chemical compounds including the

chemical carcinogens found in cigarette smoke. Aryl hydrocarbon hydroxylase (AHH) is a part of the cytochrome P-450 containing microsomal enzyme system that is present in several tissues of humans and many animal species. The activity of this enzyme system is induced following exposure to the appropriate chemical stimulus. The hydroxylation of polycyclic hydrocarbons results in the detoxification of some and the activation of others to reactive carcinogenic forms. An understanding of the role of AHH in the metabolism of chemical carcinogens in man may help clarify some of the mechanisms involved in tobacco carcinogenesis. Recently, several studies examined AHH activity in animals and man.

## Studies in Animals

Sydnor, et al. (*88*) found that an aqueous extract of CSC administered in the drinking water of rats potentiated benzo(a)pyrene-induced AHH activity in the liver. The liver AHH activity was slightly increased by the aqueous extract of CSC alone.

Rondia and Gielen (*75*) reported that rats exposed to various levels of carbon monoxide developed a decrease in AHH activity in liver homogenates. The reduction in AHH activity developed after 120 hours exposure to levels of carbon monoxide which produced carboxyhemoglobin levels below 15 percent.

Welch, et al. (*99*) reported that the administration of benzo(a)pyrene to pregnant rats resulted in an increase of the in vitro AHH activity of maternal liver, placenta, and fetal liver. A twentyfold higher dose of benzo(a)pyrene was necessary for stimulation of AHH activity in fetal liver than in the placenta or maternal liver.

## Studies in Man

Levin, et al. (*52*) studied the induction of AHH activity in human skin. Human foreskin obtained from circumcised children was maintained in tissue culture medium. Exposure to 10 $\mu$/M. of benzo(a)pyrene for 16 hours led to a twofold to fivefold increase in the activity of AHH in the exposed skin over control values.

Whitlock, et al. (*101*) reported the presence of AHH in human lymphocytes. The AHH activity of lymphocytes compared to rat liver or hamster embryo cells is relatively low. Treatment with pokeweed mitogen alone increased AHH activity about twofold. However, a threefold to eightfold greater AHH activity was found in cells treated with the mitogen and benz(a)anthracene than in resting cells.



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In studies of tobacco carcinogenesis, cigarette smoke condensate (CSC), subfractions of CSC, and individual chemical compounds found in CSC have been administered to a variety of animals using several routes of administration. Tests on living animals are frequently complicated and time consuming. Cell and tissue culture systems offer an alternate tool for the study of carcinogenesis which, in some instances, is relatively more rapid than animal testing. Specific enzyme systems and other cellular functions can often be studied in greater detail using these systems. Cells obtained from a variety of tissues and animals can be grown or maintained in culture bottles when nourished with an appropriate nutritive medium in a supportive atmosphere. When these cultures are exposed to various chemical compounds, changes can occur which may range from minor morphologic variations to malignant transformation or cell death. Toxic effects on cell cultures must be differentiated from malignant transformation. Several studies have recently examined the effect of cigarette smoke condensate or individual polycyclic hydrocarbons found in CSC on various cell and tissue culture systems.

Benedict, et al. (*4*) studied polycyclic hydrocarbon produced cytotoxicity, malignant transformation, and chromosome deformity in a variety of cell lines derived from rats, hamsters, and human tumor cells. The cytotoxic effect of benzo(a)pyrene was found to be related to the aryl hydrocarbon hydroxylase activity (AHH) of the given cell culture. Benzo(a)pyrene was cytotoxic to fetal rat hepatocytes, but this effect was probably related to the action of the hydroxylated metabolite, 3-hydroxybenzo(a)pyrene, since the cytotoxicity was blocked when the AHH system was overloaded with phenobarbital. Cell strains not possessing AHH activity showed no cytotoxic effects from benzo(a)pyrene alone; however, in the presence of fetal rat hepatocytes possessing AHH activity, enough benzo(a)pyrene metabolites were secreted into the medium to induce cytotoxic effects in the normally resistent cell lines. In hamster secondary cultures, at the chromosome level cytotoxicity was associated with chromatid breaks, whereas malignant transformation was more closely related to aneuploidy.

Diamond (*19*) studied the metabolism of benzo(a) pyrene and 7,12-dimethylbenz(a)anthracene (DMBA) in mouse, hamster, rat, monkey, and human cell cultures. Metabolism of hydrocarbons to "alkali soluble" and "water soluble" derivatives was measured. The results suggested that the parent compounds were first metabolized to "alkali extractable" derivatives and then to "water soluble" derivatives. All the cell cultures tested which were sensitive to the growth-inhibitory effects of benzo(a)pyrene or DMBA were able to metabolize these

carcinogenic hydrocarbons to "water soluble" derivatives. The data are consistent with the hypothesis that metabolism of the carcinogen is required for growth-inhibitory or cytotoxic effects.

Several authors have examined malignant transformation in cell cultures. Inui and Takayama (*41*) cultured hamster lung fibroblasts and then exposed them to crude cigarette "tar" for a period of 3 hours. Between 2 to 48 hours following this exposure, toxic effects of the "tar", including cell necrosis, swelling, vacuolization, and disintegration of cytoplasm were observed. The death of 40 to 70 percent of the cells within 72 hours was followed by the appearance of transformed cells which grew at rapid rates. These transformed cells produced malignant tumors when inoculated in the cheek pouch of hamsters. Control cell lines produced no changes when inoculated in a similar fashion.

In a similar study by Di Paolo, et al. (*21*), transformation of primary hamster cell cultures was induced by benzo(a)pyrene, 3-methylcholanthrene, or 7,12-dimethylbenz(a)anthracene. Transformed cell lines were established and subsequently inoculated in hamsters producing malignant tumors at various sites. Characteristic chromosomal changes in the transformed cells were also described.

An increase in proliferation and tumor production rate of L-Strain cells produced by treatment with cigarette "tar" was studied by Inui and Takayama (*40*). L-Strain cell cultures not exposed to "tar" did not produce tumors when inoculated in C3H mice. After an exposure to low concentrations of cigarette "tar" significant changes occurred in the cultures characterized by enlarged cells with vacuolated cytoplasm, giant cell formation, and accelerated growth rates. These transformed cells produced tumors in 70 percent of injected C3H mice.

Nagata (*60*) treated cell cultures obtained from kidneys of newborn mice with 20-methylcholanthrene in various concentrations. Control cultures could not be maintained for long; however, the treated cells formed two permanent cell lines which had a transformed morphology and altered karyotypes. Epithelial carcinomas were produced after the subcutaneous injection of these transformed cells into unconditioned newborn mice.

Freeman, et al. (*29*) isolated hamster-specific C-type RNA viruses from tumors induced by cell cultures transformed by chemical carcinogens. Cell cultures were prepared from early passage hamster embryo cells and treated for 7 days with 3-methylcholanthrene or certain fractions of cigarette smoke condensate. Following treatment, morphologically transformed cell lines were isolated and maintained. Subsequent inoculation in newborn hamsters produced malignant tumors at the site of inoculation. New cell lines were established from some of the resulting tumors. No infectious viruses were isolated from cell lines prior to inoculation; however, C-type RNA viruses were iso-

lated from tumors and from cell lines derived from tumors. The authors concluded that the chemical treatment and activation of viruses appeared to be related events.

Sivak and Van Duuren (*83*) developed a cell culture system that responded with characteristic changes in cell morphology to the application of various fractions of tobacco leaf extracts. Certain dose-response characteristics were demonstrated, suggesting a mechanism whereby various tobacco fractions might be rapidly screened for tumor-promoting activity.

Dietz and Flaxman (*20*) studied the toxicity of aromatic hydrocarbons on normal human epidermal cells in vitro. Pieces of adult human abdominal skin were maintained in tissue culture medium and exposed to 3-methylcholanthrene and benzo(a)pyrene at a concentration of 1 μg./ml. for a period of 4 days. The cultures were then kept for an additional 3 months following exposure. No malignant transformation occurred; however, giant cells and a more disorderly pattern of growth were observed in the treated cultures weeks earlier than similar changes in control cultures.

### *Binding of Polycyclic Hydrocarbons to DNA and RNA*

There is evidence that some chemical carcinogens including certain of the polycyclic hydrocarbons found in cigarette smoke condensate are active because of the reaction of the carcinogen or a reactive metabolite with cellular macromolecules. Duncan, et al. (*23*) studied a series of radioactive polycyclic hydrocarbons with respect to their metabolism and tendency to bind with cellular DNA and RNA in monolayer cultures of primary mouse embryo cells. All the tested hydrocarbons were metabolized to "water soluble" metabolites at approximately equal rates. A "binding index" was calculated to determine the binding of various hydrocarbons to cellular DNA and RNA. The group of hydrocarbons with a high "binding index" consisted of potent carcinogens, while another group with much lower values for the "binding index" were with but one exception non-carcinogens.

Carlassare, et al. (*12*) studied the in vivo binding of benzo(a)pyrene to DNA. Benzo(a)pyrene-$^3$H was fed to male and female NCL mice which were sacrificed after 15 hours. The DNA was extracted and purified from the skin, spleen, and liver. The binding of benzo(a)pyrene was greatest in the liver and somewhat less in the spleen and skin. It was calculated that the average molecular weight of DNA was 6 million and that 1 molecule of benzo(a)pyrene was bound to every 46.8 molecules of DNA in the liver, suggesting covalent binding of benzo(a)pyrene to DNA.

Alexandrov and Vendrely (*1*) found that cigarette smoke condensate, the hexane-extracted fraction of CSC, and benzo(a)pyrene all inhibited RNA synthesis in mouse skin.

### *N-Nitrosamines in Tobacco Smoke*

The largest number of chemical carcinogens which have been identified in cigarette smoke condensate are polycyclic hydrocarbons. N-nitrosamine compounds known for many years to be potent carcinogens have produced malignant tumors in a number of organ systems of a wide variety of animals. These compounds were recently identified in cigarette smoke. Only recently has an association been found between exposure to N-nitrosamines and malignant tumors in humans (*55*). N-nitrosamines are formed chemically by a reaction of NO and $NO_2$ or nitrites with secondary amines. The chemical precursors of the N-nitrosamines have been identified in cigarette smoke condensate (CSC) by a number of investigators. These studies were reviewed by Wynder and Hoffmann (*102*). More recently, Rhoades and Johnson (*69*) developed a method for the determination of N-nitrosamines in tobacco smoke condensate using gas chromotography. Two N-nitrosamines were found in CSC: one was identified as N-dimethylnitrosamine (DMNA) and the other was believed to be N-methylethylnitrosamine (MENA) (*43*, *70*). The concentration of DMNA per cigarette in nanograms was determined in condensates from experimental cigarettes made from single tobacco varieties rather than a tobacco blend. Each tobacco tested was grown in both a low- and high-nitrogen soil. High-nitrogen soil conditions resulted in a considerable increase in nitrosamines. A popular brand of nonfilter cigarettes was also tested. These results are presented in table 2.

TABLE 2.—*N-dimethylnitrosamine (DMNA) content of condensates obtained from several tobaccos grown in both "high" and "low" nitrogen soils*

| Tobacco type | Soil nitrogen | DMNA (nanograms per cigarette) |
|---|---|---|
| Robinson | Low nitrogen | 0 |
| Catterton | do | 5 |
| Burley | do | 3 |
| Robinson | High nitrogen | 27 |
| Catterton | do | 60 |
| Burley | do | 140 |
| U.S. nonfilter | | 8 |

Source: Johnson, D. E., Rhoades, J. W. (*45*).

## Summary of Recent Cancer Findings

In addition to the summary presented in the introduction of this chapter, based on previous reports of the health consequences of smoking, the following statements are made to emphasize the recent developments in the field:

1. Recent epidemiological and autopsy studies from several countries confirm that cigarette smoking is the major cause of lung cancer.
2. Continued cigarette smoking by patients following successful surgical removal of a cancer of the oral cavity, pharynx, or larynx without tumor recurrence for a period of 3 years is associated with a significant increase ($P < 0.001$) in the risk of developing a second primary cancer of the upper respiratory or digestive tract compared to similar patients who discontinue smoking at the time of their surgery.
3. The intratracheal administration of certain polycyclic hydrocarbons found in cigarette smoke condensate results in the formation of anaplastic and squamous cell cancers of the lung and respiratory tract in hamsters and rats. Many of these tumors are histologically similar to the lung cancers found most frequently in cigarette smokers.
4. The application of cigarette smoke condensate or polycyclic hydrocarbons to various cell cultures often results in transformation to cells with a more rapid and disorderly growth pattern. Transformed cell lines frequently produce benign or malignant tumors when transplanted to experimental animals.
5. N-nitrosamines have been identified in cigarette smoke. These compounds are known to be potent cancer causing chemicals for a variety of animals. They appear to be formed in higher concentrations from tobaccos raised under high-nitrogen soil conditions.

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# CHAPTER 4

## Pregnancy

# Contents





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